placing it either in other plasmids for expression level analysis (E1-Mogy, 2012) or within the E1 region of the Adenovirus genome subject to the insertion of sites to regulate its expression (Molin, 1998). Other work has included the mutation of the MLP TATA box for both basic research (Concino et al., 1983; Concino et al., 1984) and in order to allow the virus to be selectively grown only in cells which have a trans-complementing factor in a cell. Importantly, the latter approach provides for a system where the activity of the major late promoter is entirely dependent on a complementing protein factor being present in a cell, and therefore in a cell where this factor is absent the MLP is not active. The invention, however, provides the converse of this, where the MLP maintains it full expression activity level in cells where a repressor not bound, providing high level virus replication with minimal disturbance to the virus life cycle.

[0014] The virus of the invention described herein is therefore fully active when not repressed but is capable of being repressed, depending on the presence or absence of a repressor. A repressor binding site has not previously successfully been inserted into the MLP in situ for the regulation of its expression in an adenovirus genome. The current inventors have further improved this system to place the repressor protein coding sequence under the control of the Major Late Promoter itself. In this approach, the Major Late Promoter self-represses itself because when the Major Late Promoter tries to transcribe the structural proteins of the virus, it will also transcribe a repressor capable of repressing its own activity, thereby allowing for a negative feedback loop that prevents MLP activity and providing tight regulation of MLP expression.

[0015] It is therefore an object of the invention to provide an adenoviral vector system which provides increased yields of recombinant proteins, and can be used to provide non-adenovirus virus and virus-like particles.

[0016] It is also an object of the invention to provide a process of producing a transgene product in adenovirus-infected cells, which reduces the need to remove virus particles from the purified product which may be a protein, non-adenovirus particle or virus-like particle.

DESCRIPTION OF THE DRAWINGS

[0017] FIG. **1** shows a schematic diagram of the adenovirus genome and shows the main early and late expression regions. The MLP is also shown. Splice patterns are denoted by dotted lines.

[0018] FIG. **2** shows annotated sequences (SEQ ID NOs: 12-14) of the major late promoter region of wild type adenovirus serotype 5.

[0019] FIG. **3** shows annotated sequences (SEQ ID NOs: 15-17) of the modified major late promoter region of adenovirus serotype 5.

[0020] FIG. **4** shows schematic diagrams of plasmid vectors constructed for expression of the GFP reporter from the Ad5 repressor mutant Major late promoters.

[0021] FIG. **5** shows plasmid vectors expressing the GFP reporter gene from MLP promoters.

[0022] FIG. **6** shows transcriptional repression of the repressor mutant MLP by the TETR protein.

[0023] FIG. **7** shows examples of MLP repressor Ad5 in repression in HEK293 T-Rex Flp cells, a cell line stably expressing the TETR protein.

[0024] FIG. **8** shows examples of MLP repressor Ad5 in repression in 293Ad cells transfected with a TETR expression plasmid (pTETR), under the control of the constitutive CMV (cytomegalovirus) promoter.

[0025] FIG. **9** shows viral genome replication of repressed MLP mutant Ad5 in 293 T-Rex Flp cells, a cell line stably expressing the TETR protein.

[0026] FIG. **10** shows Ad5 with TET1 b modified MLP variants infection of HEK293 cells at low MOI. A) HEK293 cells were transduced with E1/E3 Ad5 or Ad5 TET1 b MLP expressing EGFP under control of the CMV promoter at MOI 0.1 and in the presence doxycycline 0.2 μg/mL or DMSO. Image of day-6 post infection by fluorescence microscopy. HEK293 cells were also harvested for flow-cytometry analysis to determine the percentage of EGFP positive cell within the gated population (B). Data as mean±SD of triple biological repetition.

[0027] FIG. **11** shows EGFP expression from Ad5 with TET01b modified MLP. A) Plasmid expression of EGFP encoding Ad5 TPL from the CMV promoter. HEK293 cells transfected with plasmid DNA expressing EGFP from a promoterless plasmid or under control of the CMV promoter with and without the Ad5 TPL. EGFP expression determined by flow cytometry 48 h post-transfection. B) MLP TET01b modified Ad5 expressing EGFP (with and without Ad5 TPL) under control of the CMV promoter in T-Rex Flp cells treated with DMSO or doxycycline 0.2 ug/mL. Cells analysis by flow cytometry 24 h, 48 h and 72 h post-infection. C) EGFP expression under control of the CMV promoter from plasmid DNA or MLP modified Ad5 in HEK293. HEK293 cells were transfected with plasmid expressing TETR prior to transduction with the CMV promoter plasmid expressing EGFP or MLP modified Ad5 expressing EGFP (with and without the Ad5 TPL) under control of the CMV promoter, in the presence of doxycycline or DMSO. Cells were harvested for flow cytometry 48 h post-transduction. D) Fluorescent microscopy image of pTETR transfected HEK293 cells transduced with MLP modified Ad5 expressing EGFP encoding Ad5 TPL, in the presence of DMSO or doxycycline 0.2 μg/mL. Image 48 h post-infection. MFI (median fluorescent intensity). Data as mean±SD.

[0028] FIG. **12** shows Western blot detection of adenovirus structural proteins from TERA-CMV-EGFP or E1/E3 Ad5-CMV-EGFP infection in HEK293 cells. A) TERA or E1/E3 Ad5 was used to transduce HEK293 at MOI 100 or 1000. Virus were harvested from cell lysate (left blot) or growth medium (right blot). B) Western blot analysis of growth media from HEK293 cells cultured in a dose escalation of doxycycline and transduced with TERA or E1/E3 deleted Ad5 at MOI 10 or MOI 100 (C). All virus samples harvested at 72 h post-infection and detected using anti-Ad5 antibodies with automated Western machine (Wes Simple Western).

[0029] FIG. **13** shows DNA and virus replication of TERA in HEK293 cells. A) HEK293 cells were infected with TERA or E1/E3 Ad5, encoding the CMV EGFP expression cassette, at MOI 1, 10 or 100, and total DNA was harvested at the indicated timepoint post-infection for QPCR analysis. B) TERA or E1/E3 Ad5, encoding the CMV EGFP expression cassette, was used to infect HEK293 at MOI 10 or 100, in the presence of doxycycline 0.5 μg/mL or DMSO. Total DNA was harvested at the indicated timepoint post-infection for QPCR analysis.